AWARD NUMBER: W81XWH-14-1-0579

TITLE: Targeting Epigenetic Mechanisms in Pain due to Trauma and TBI

PRINCIPAL INVESTIGATOR: David J. Clark, MD

RECIPIENT: Palo Alto Veterans for Research and Education, Palo Alto, CA 94304

REPORT DATE:

October 2017

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2017	Annual	30 Sept 2016- 29 Sept 2017

4. TITLE AND SUBTITLE
Targeting Epigenetic Mechanisms in Pain due to Trauma and TBI

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0579

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
David J. Clark, MD

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
VA Palo Alto Health Care System/PAVIR
3801 Miranda Ave
Palo Alto, CA 94304

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Persistent pain after TBI, trauma to the extremities and in the situation where both types of injury exist is highly problematic. For example, persistent pain after surgery and other forms of soft tissue injury occurs in up to 50% of patients, and as many as 85% of those with TBI experience ongoing pain. Battlefield trauma, motor vehicle accidents and sports-related injuries are particularly likely to involve TBI, peripheral trauma or both. Disability due to pain and other causes is very high amongst such patients. We have no effective approaches to reducing the likelihood of developing chronic pain after TBI or peripheral injuries, and the mechanisms supporting such pain are poorly understood. Recent advances have suggested, however, that epigenetic changes occurring in the dorsal horn of the spinal cord after either brain or peripheral trauma may support chronic pain. Our work to-date has established a rodent model of TBI in combination with injury to a limb as a model for addressing this clinical problem. We have established the severity and time course of pain-related changes after TBI and incision. Critically, we have demonstrated that histone deacetylase inhibitors greatly exacerbate the pain problems while agents that block histone acetylation reduce the pain-related changes. Additional evidence suggests that changes in the levels of genes in the spinal cord along with brain-level changes after TBI may be responsible. These observations suggest novel approaches to treatment.

15. SUBJECT TERMS
Traumatic Brain Injury, Chronic Pain, Epigenetic, Chemokine, Disability, Analgesia, Spinal Cord

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	20	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)

Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:**

Persistent pain after TBI, trauma to the extremities and in the situation where both types of injury exist is highly problematic. For example, persistent pain after surgery and other forms of soft tissue injury occurs in up to 50% of patients, and as many as 85% of those with TBI experience ongoing pain. Battlefield trauma, motor vehicle accidents and sports-related injuries are particularly likely to involve TBI, peripheral trauma or both. Disability due to pain and other causes is very high amongst such patients. We have no effective approaches to reducing the likelihood of developing chronic pain after TBI or peripheral injuries, and the mechanisms supporting such pain are poorly understood. Recent advances have suggested, however, that epigenetic changes occurring in the dorsal horn of the spinal cord after either brain or peripheral trauma may support chronic pain. Specifically, the acetylation of histone proteins with spinal cord dorsal horn neurons leads to the sustained up-regulation of pain-related chemokine receptor CXCR2 thereby supporting chronic pain. The objective of this project is to define the role of agents targeting epigenetic mechanisms in reducing pain and disability after trauma, particularly in the setting of TBI. This objective is closely in alignment with the pain management focus area of the CRMRP Neurosensory Research Award program. Specifically, these studies involve, 1) applied research on alternative non-opioid analgesic drugs, 2) strategies for management of acute and chronic pain under the care of a clinician in non-deployed settings (specifically in patients with TBI), and 3) research studies to evaluate novel analgesics and mechanisms of pain in relevant animal models. At the completion of the proposed studies we will have addressed our project's main objective using multiple approaches. We will have a refined mechanistic understanding of how tissue trauma, TBI and the combination lead to the experience of chronic pain. We will also have preclinically evaluated the complementary approaches of using HAT or chemokine signaling inhibition to reduce chronic pain and disability after TBI and soft tissue trauma.

2. **KEYWORDS:** Traumatic Brain Injury, Chronic Pain, Epigenetic, Chemokine, Disability, Analgesia, Spinal Cord

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

*Please note our request for extension of the project was just approved. The timelines and plans reflect the submitted plans for the fourth year where appropriate.

The approved project was accompanied by a Gantt chart listing major specific tasks (ST's). The headings below refer to those tasks. The following summary of major goals reflects the status of the project to-date.

Specific Aim 1: To evaluate the hypothesis that histone acetyl transferase (HAT) inhibitors reduce pain and disability after surgical incision, TBI and the combination of the two injuries.

Major Task 1 (Pre-experimental animal approval)

ST1.1 Local IACUC Approval:
Complete

ST1.2 DoD ACURO Approval:
Complete

Major Task 2: Establish the roles of HAT inhibitors on simple measures of nociception after incision and TBI.

ST2.1 Measure effects of HAT inhibitors on nociceptive sensitization after incision.
Complete

ST2.2 Measure effects of HAT inhibitors on nociceptive sensitization in TBI model.
Complete

ST2.3 Measure effects of HAT inhibitors on nociceptive sensitization after incision, tibia fracture and TBI.
75% Complete

ST2.4 Test effective doses of drugs in open field and rotarod paradigms.
Complete

Major Task 3: Establish the roles of HAT inhibitors on more complex pain and functional measures as well as the efficacy of oral preparations of HAT inhibitors after incision and TBI.

ST3.1 Measure effects of HAT inhibitors on complex pain behaviors (CPP, PGE2) and gait changes after incision and TBI.
60% Complete

ST3.3 Measure the efficacy of curcumin on pain, cognitive and mood in a mouse closed-head model of TBI +/- limb fracture.
Complete

Specific Aim 2: To evaluate the hypothesis that HAT inhibitors block incision-related epigenetic histone acetylation in control and TBI model animals thereby normalizing expression of key pain-related genes.

Major Task 4: Identify the type and cellular location of epigenetic changes in spinal cord tissue after incision and TBI, and the relationship of those changes to CXCR2 expression.

ST4.1 Establish spinal cord sites and cell types displaying enhanced histone acetylation (AcH3K9) in the settings of incision/TBI.
70% Complete

ST4.2 Establish spinal cord sites and cell types displaying enhanced CXCR2 expression and co-localization with histone acetylation in the settings of incision/TBI.
70% Complete

ST4.3 Establish the role of descending facilitation in supporting nociceptive and spinal molecular changes after TBI+limb fracture in mice and rats.
50% Complete

Major Task 5: Identify changes in spinal cord HAT activity and the consequences of those changes in terms of reducing pain and functional impairment after incision and TBI.

ST5.1 Measure changes in spinal cord HAT activity hypothesized to be caused by hindpaw incision, TBI or the combination. Determine if blockade of HAT activity reduces incision, TBI and incision/TBI-induced increases in the expression of spinal cord CXCR2.

Complete

Major Task 6: Examine specifically the efficacy of a selective CXCR2 antagonist in reducing pain and functional impairment after incision and TBI.

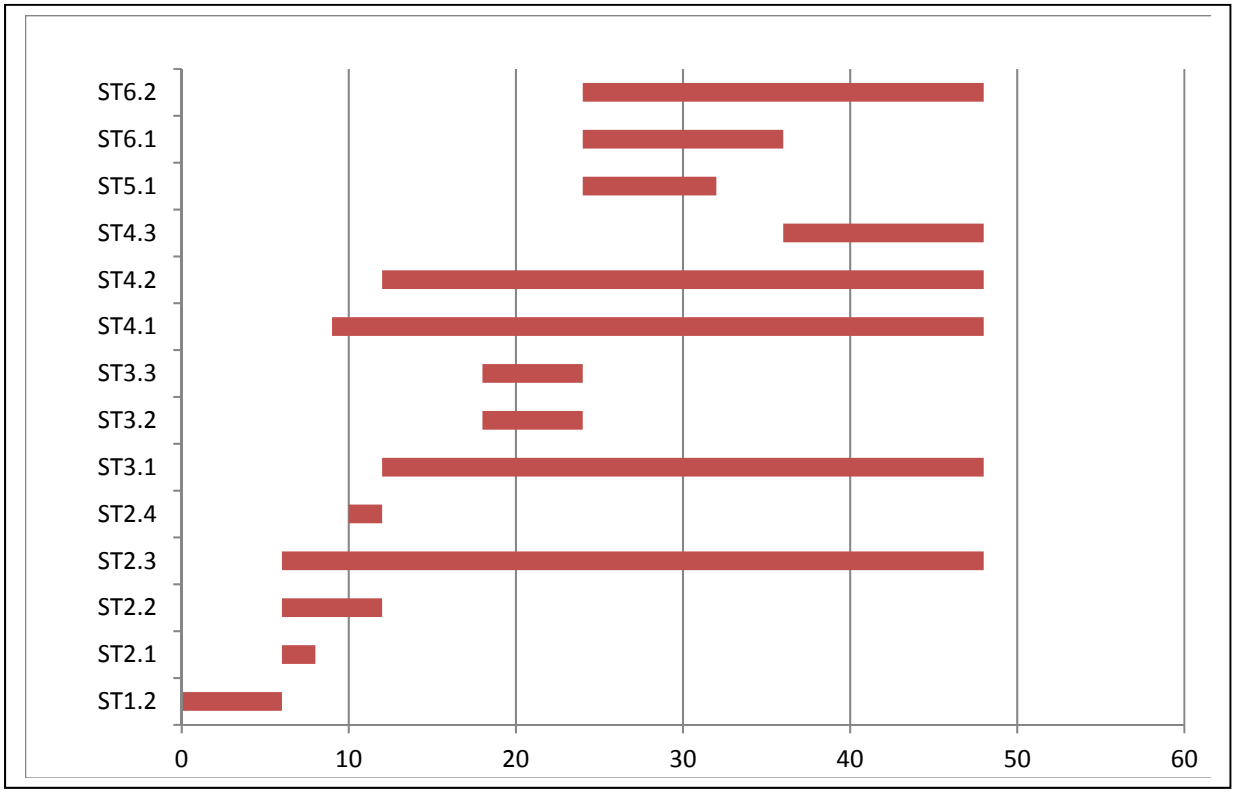
ST6.1 Determine the efficacy of selective CXCR2 antagonists in reducing pain-related behaviors in the incisional, TBI and combination models.

Complete

ST6.2 Determine the efficacy of selective CXCR2 antagonists in reducing pain-related behaviors in the mouse TBI and combination fracture/TBI mice.

60% Complete

Gantt chart for project. Note the Y-axis lists the subtasks, and the X-axis displays the project months.



What was accomplished under these goals?

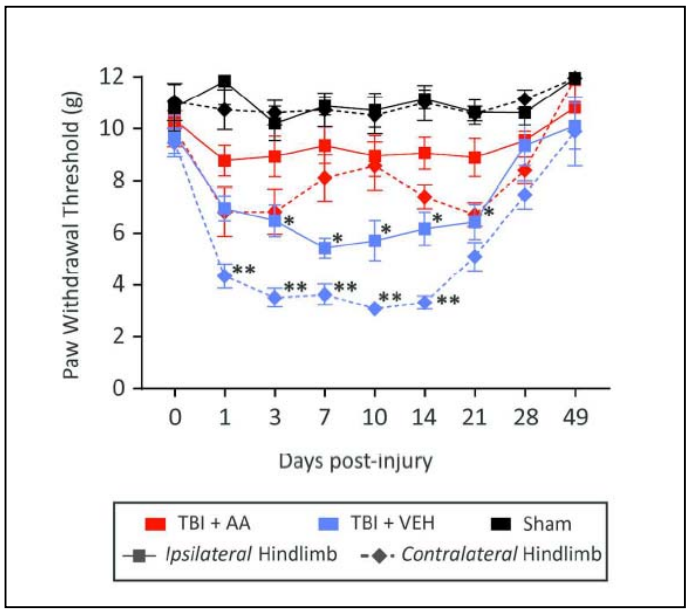
The following section of the report focuses on the accomplishments of the previous year including the outcome of studies presented as partially complete in the previous annual report. This has been a very productive year for the project with full length publications accepted or submitted recently. Major findings are presented in graphical form with descriptions of additional accomplishments in text format.

Rat TBI model:

(The following studies support Specific Aim 1, Major Task 2)

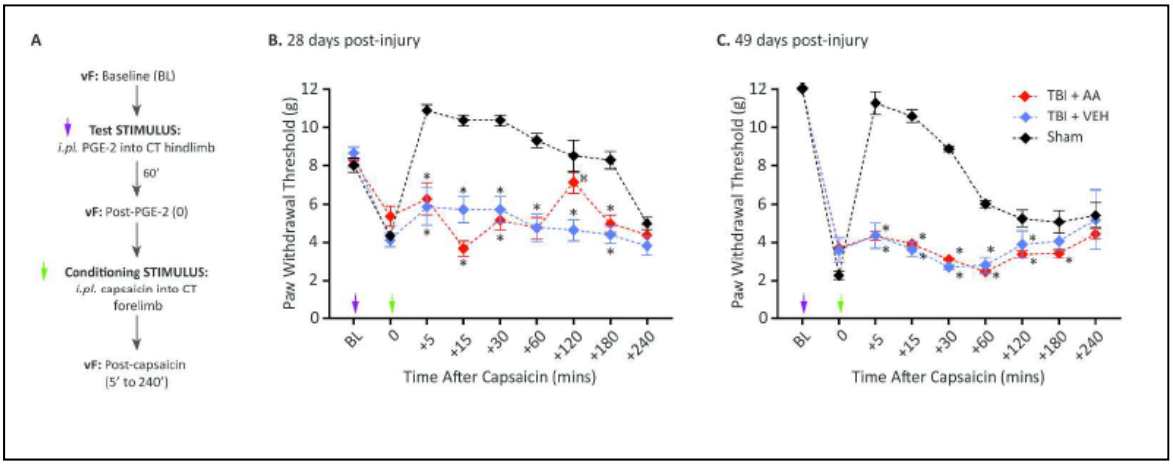
A major goal of the project involves the use of the rat LFP TBI model in which to study the effects of TBI on pain-related behaviors as well as the effects of the histone acetyltransferase (HAT) inhibitor anacardic acid (AA). Histochemical and immunohistochemical staining was used to examine the structural underpinnings of the effects. In addition, we looked at effects both 7 days after TBI when AA effects were maximal, and 28 days when allodynia resolves in TBI animals but latent sensitization remains suggesting more persistent changes in CNS functioning after TBI. The studies described below have been submitted for publication (Ferguson et al., 2017). A few of the key results are presented and discussed below.

To clearly demonstrate the effects of AA on pain sensitization, we gave AA to rats beginning immediately after TBI and continuing once daily for 7 days. Vehicle was given to other animals, and sham controls were used. The results presented below demonstrate that AA substantially prevented mechanical pain sensitization for the first several weeks after injury.

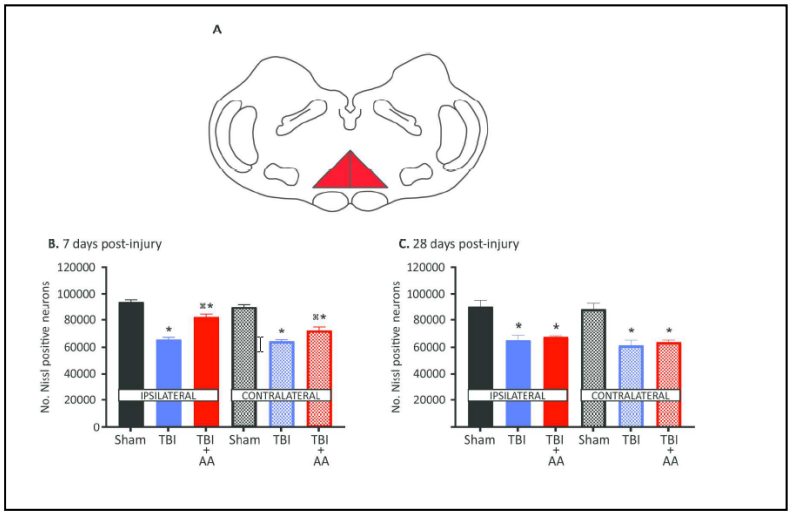


(The following studies support Specific Aim 2, Major Tasks 4)

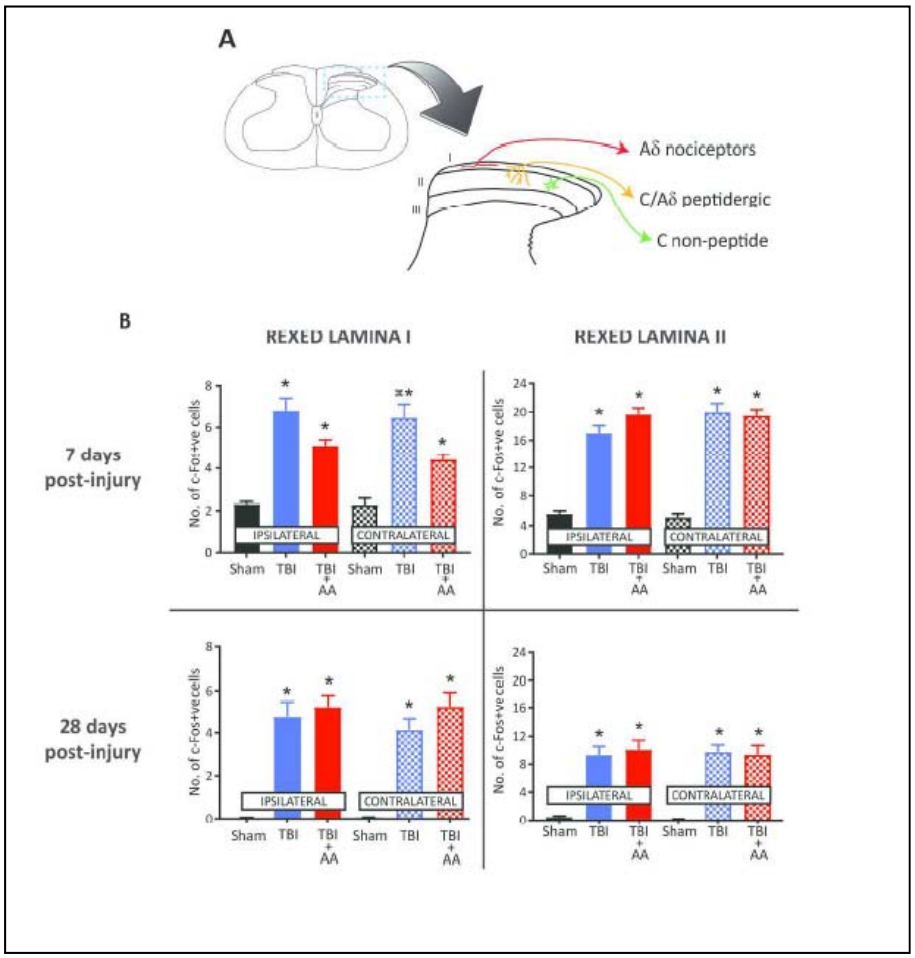
While it was clear that AA prevents much of the initial pain sensitization observed in this model, we know that pain after TBI can be very problematic in its chronic form. Therefore, we measured the efficiency of descending inhibitory pain control in rats after recovery from sensitization. Deficient control of this type is strongly linked to chronic pain. The graphs below show that our protocol for measuring descending control of pain is robust in sham treated rats either 28 or 49 days from injury, but that TBI animals showed completely abolished descending pain control whether or not they were treated with AA immediately after TBI. Thus, AA is an excellent early phase analgesic, but one that cannot prevent longer term pain sensitization if given short term.



To determine why we saw positive effects of AA at early time points, but no prevention of loss of descending inhibition 4 weeks after TBI, we examined the effects of AA on various measures of damage to CNS tissues at both early (7d) and late (28d) time points. These points were chosen as days on which AA was having strong effects (7d), or loss of effect (28d) after TBI. We examined cortical, subcortical, brainstem and spinal cord tissues. Below we first present data on cell loss in the RVM, a major descending regulatory control center in the brainstem that projects to the dorsal horn of the spinal cord to control the flow of nociceptive information. The results show that AA partially prevented cell loss in this center 7d after injury, but these effects were lost by 28 days.

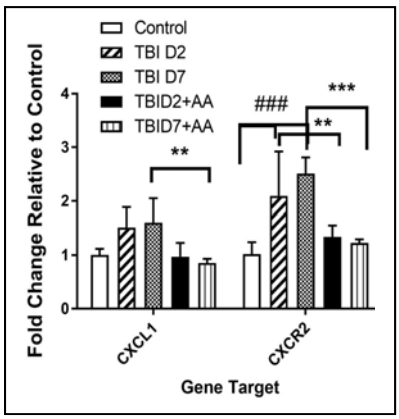


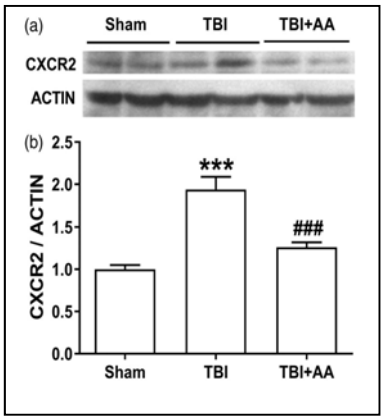
We went on to look at changes in the expression of Fos, a marker of neuronal activation and surrogate for activation of nociceptive neurons in spinal cord tissue. As can be seen in the figure below, we again observed some effects of AA to prevent TBI-induced changes 7d after injuries, but these effects had disappeared by 28 days after injury. Together we feel that these and our other data demonstrate that the short term suppression of HAT activity after TBI may moderate short term effects, but that long-term changes will require a more aggressive approach.

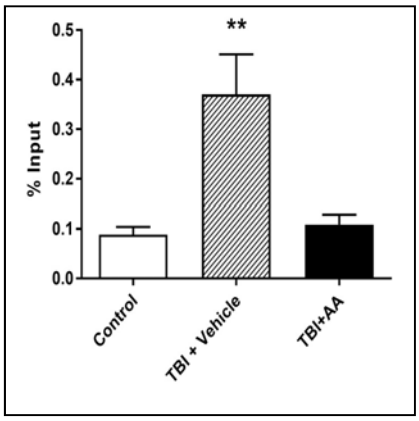


(The following studies support Specific Aim 2, Major Tasks 4 and 5)

We previously reported the preliminary results of our studies on CXCR2 signaling in spinal cord tissue after TBI, and those results have now been published (Liang et al., 2017). One key set of findings finalized during the past year was the establishment of epigenetic control of CXCR2 expression after TBI. We conducted CXCR2 expression studies on lumbar spinal cord tissue contralateral to the side of TBI (more sensitized). We found that CXCR2 was expressed at higher levels after TBI, and that the histone acetyltransferase (HAT) inhibitor arachidonic acid blocked that expression 2 and 7 days after TBI. These results were obtained for both mRNA and protein measurements. Key to the central hypothesis of the project were the very technically challenging and time consuming ChIP studies demonstrating that TBI in fact increased the association of acetylated histone protein with the CXCR2 promoter, and the blockade of that association with an inhibitor of HAT activity.

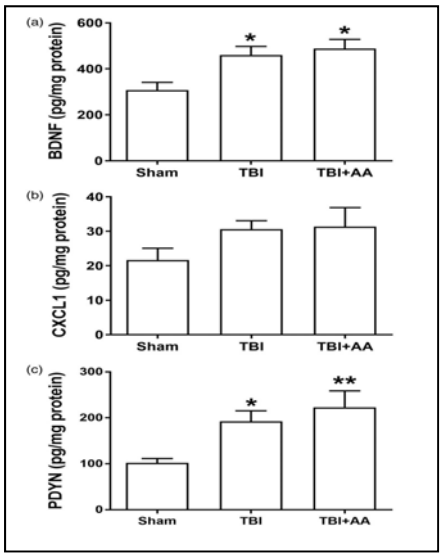






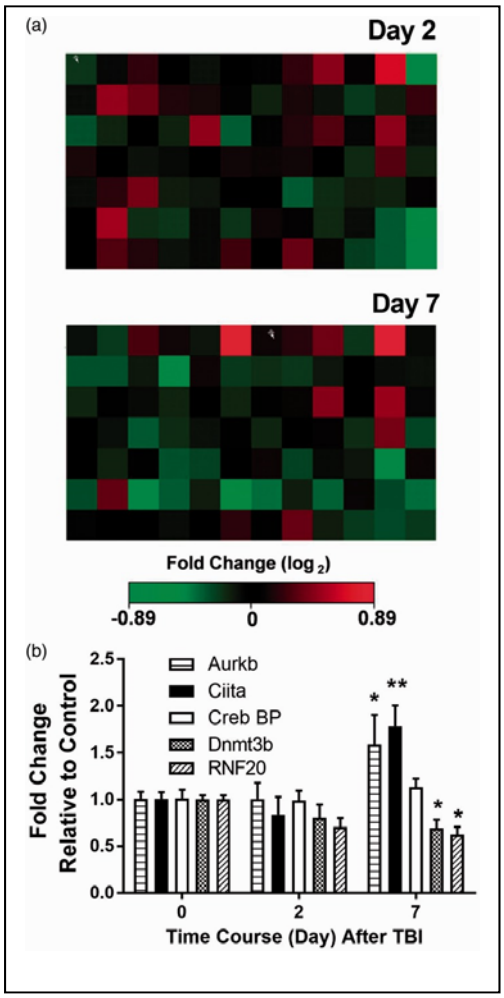
(The following studies support Specific Aim 2, Major 5)

We were able to go on to demonstrate that this HAT-mediated regulation was not generalizable to all up-regulated spinal cord genes after TBI. Using ELISA assays we showed that BDNF, CXCL1 and prodynorphin levels in spinal cord tissue were indeed elevated after TBI, but were not reduced by anacardic acid treatment as shown below.



(The following studies support Specific Aim 2, Major Task 5)

In addition to the pharmacological characterization of HAT activity and effects on gene expression, an important task for the project was to identify the activation or changes in expression of epigenetically-related genes in spinal tissue after TBI. To that end we conducted a series of expression array experiments on spinal cord tissue at 2 time points after TBI. Those results showed the selective change in expression of only 4 epigenetic genes. One of the up-regulated genes, Ciita, is a known regulator of HAT activity thus providing significant mechanistic information to the project's results.



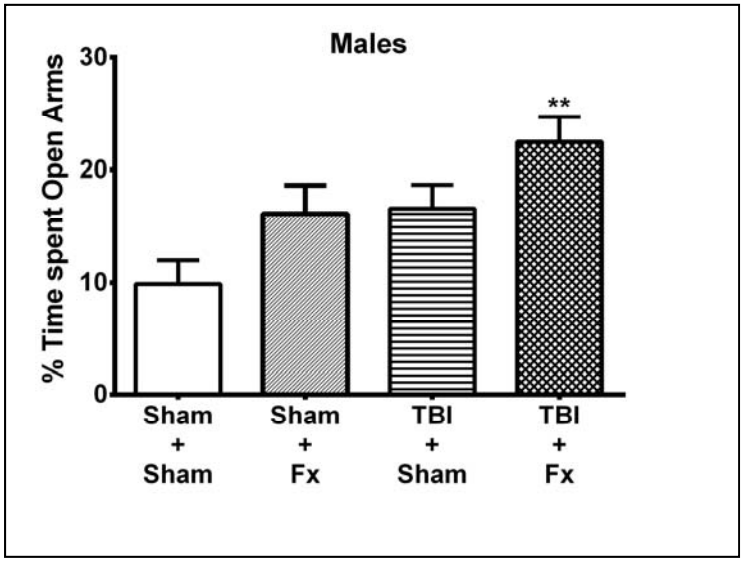
Mouse TBI model:

In the year 2 annual report we provided data from mouse TBI and limb fracture/TBI polytrauma subjects characterizing that model and the interactions of TBI with peripheral trauma. We have since completed those experiments and submitted a paper showing:

1. In mice, closed head TBI leads to bilateral lower extremity sensitization lasting about 2 weeks (Specific Aim 1, Major Task 3).
2. TBI and tibial fracture simultaneously leads to a model in which recovery from mechanical nociceptive sensitization is prolonged (Specific Aim 1, Major Task 3).
3. TBI and tibial fracture worsen memory-related outcomes compared to fracture alone (Specific Aim 1, Major Task 3).

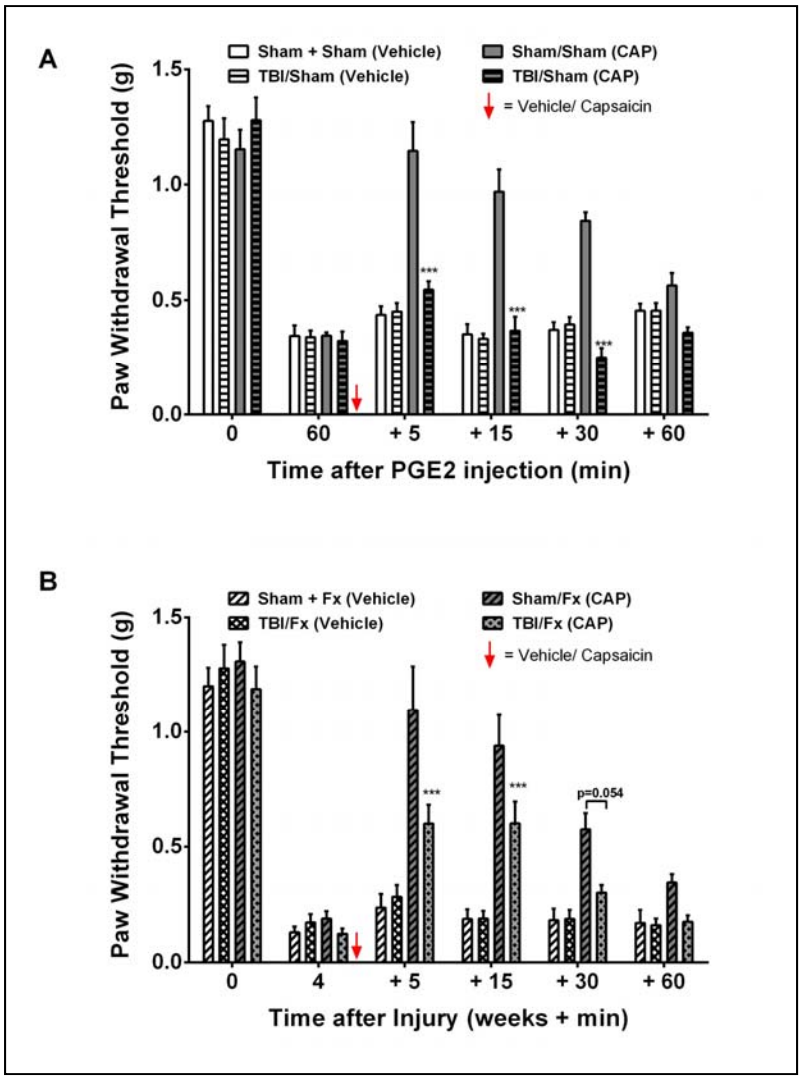
Over the past year, we expanded the mouse TBI and polytrauma dataset, and have submitted a manuscript for publication (Sahbaie et al., Submitted). The principal additional data collected to complete that manuscript includes the measurement of an additional behavior, time spent in the open arms of a zero maze for male mice, as a measure of anxiety. As shown below, TBI plus fracture led to effects more serious than either injury individually.

(The following studies support Specific Aim 1, Major Task 3)



(The following studies support Specific Aim 2, Major Task 4)

In addition, we have performed critical experimentation showing that descending inhibition, a major mechanism responsible for suppressing chronic pain implicated in TBI-related pain conditions, is almost completely lost after TBI or TBI and limb fracture. This is important information, as therapies such as with newer SSRI-class antidepressants might help patients with this particular type of deficit. In panel A below, mice having sustained mild closed head TBI have their hindpaws sensitized by the injection of PGE2. Descending inhibition is then assessed by injecting a single forepaw with capsaicin. Positive descending inhibition of pain is measured as the increase in withdrawal threshold observed in the previously sensitized hindpaw. In panel B we display data from the fracture-sensitized hindpaws of fracture-TBI and fracture alone mice. In this case, forepaw capsaicin injection reversed sensitization in the fracture alone animals, but not those with TBI. Again, this has implications for the treatment of pain in patients after polytrauma.



What opportunities for training and professional development has the project provided?

The project was not generally designed to provide professional development opportunities. However, the learning of new methods and familiarization of staff with a new area of science and medicine does represent a benefit of the work completed.

What do you plan to do during the next reporting period to accomplish the goals?

*Please note that a request for extension of the project for a fourth year was just approved by CDMRP.

The funds will be used for several groups of experiments outlined below. The principal associated costs will be animal purchase, housing, reagents and laboratory staff salaries. The specific SOW entry is listed with the experiments.

1. SOW Aim 1/Task 2: Cohorts of polytrauma animals will be treated with HAT inhibitors or HDAC inhibitors. Nociceptive sensitization will be measured.

2. SOW Aim 1/Task 3: Cohorts of polytrauma animals will be treated with HAT inhibitors or HDAC inhibitors. Memory, gait, conditioned place preference, mood will be measured.

3. SOW Aim 2/Task 4: The spinal cords of polytrauma animals will be assessed for acetylated histone (AcH3K9), CXCR2 and CXCL1 levels.

4. SOW Aim 2/Task 4 (high-impact direct extensions of original experiments): Cohorts of polytrauma animals will be treated with 5,7 DHT, a reagent that depletes serotonin from neurons projecting from the brainstem to the spinal cord involved in descending pain modulation. The effects of this reagent on nociceptive sensitization, AcH3K9 levels and CXCR2 expression will be measured.

5. SOW Aim 2/Task 6: Cohorts of polytrauma animals will be treated with CXCR2 inhibitors, and the effects on nociception, memory, gait, conditioned place preference, mood will be measured.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Prior to this time there was very little understanding of the mechanisms linking TBI to pain, a major cause of disability after TBI. There was no explanation for why pain might be worse at sights distant from the head after TBI. This constitutes a fundamental contribution to the discipline.

What was the impact on other disciplines?

The field of pain research had very little information to this point explaining how injury to the CNS could result in pain. Here we have demonstrated that CNS injury in the form of TBI leads to fundamental changes in spinal nociceptive processing. This is a novel idea for a related set of disciplines, and helps to explain pain in other types of CNS injury and possibly neurodegenerative disease.

What was the impact on technology transfer?

It is possible that CXCR2 antagonists could be repurposed for use as analgesics after TBI.

What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:**

Publications, conference papers, and presentations (Past 1 year)
Journal publications

1. Epigenetic Regulation of Chronic Pain after Traumatic Brain Injury. De-Yong Liang, Peyman Sahbaie, Karen-Amanda Irvine, Yuan Sun, Xiaoyou Shi, Anders Meidahl, Tian-Zhi Guo, Peng Liu, David C. Yeomans and J. David Clark. *IBRO Reports* (2) June 2017, 14-23.

2. Chronic Pain after Traumatic Brain Injury: Pathophysiology and Pain Mechanisms. Karen-Amanda Irvine and J. David Clark. *Pain Medicine* (In Press).

3. Neuroprotective effects and control of TBI-induced pain by Anacardic acid. Karen Amanda Irvine, Peyman Sahbaie, De-Yong Liang, J. David Clark. *Journal of Neurotrauma* (Submitted).

4. Sex differences in nociceptive and cognitive changes in a murine model of polytrauma. Peyman Sahbaie; Maral Tajerian; Phillip Yang; Karen Amanda Irvine; Ting-Ting Huang; Jian Luo; Tony Wyss-Coray; J. David Clark. *J Pain* (Submitted).

Other publications, conference papers, and presentations

1. Characterization of nociceptive alterations and cognitive impairments in a preclinical model of polytrauma. Presented at Society for Neuroscience annual meeting at San Diego, CA on Nov. 15, 2016.

2. Sex differences in nociceptive alterations and cognitive impairments in a preclinical model of polytrauma. Presented at TBI Research Forum annual meeting at VA Palo Alto Health Care System, CA on March 31, 2017.

3. Sex differences in nociceptive alterations and cognitive impairments in a preclinical model of polytrauma. Presented at Annual Research Awards meeting at Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University, CA on May 22, 2017.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name: David J. Clark
Project Role: PI
Annualized calendar months: 2
Contribution to Project: This person is the project PI and administratively oversaw the completion of the regulatory requirements, the purchase of equipment, and the initiation of experimentation.

Name: David C. Yeomans
Project Role: Co-I
Annualized calendar months: 1

Contribution to Project: This person co-directs the experimentation. He reviews the progress of the experiments, provides scientific input and trouble-shoots scientific and technical issues.

Name: Deyong Liang
Project Role: Investigator
Annualized calendar months: 2
Contribution to Project: This person conducts the majority of the rat experimentation. He has performed the TBI surgeries as well as the incisional model. He orders the animals and plans experiments. He processes and presents the data generated.

Name: Peyman Sahbaie
Project Role: Research Associate
Annualized calendar months: 4.5
Contribution to Project: This person led the effort to acquire and set-up the TBI device. He is responsible for a portion of the animal testing, and will perform a portion of the surgeries.

Name: Karen-Amanda Ferguson
Project Role: Research Associate
Annualized calendar months: 2
Contribution to Project: This person has completed all of the neuropathological and immunohistochemical studies that are a part of this study. In addition, she will in the coming year take on the more complex animal testing protocols.

Effort listed for PI/Senior Key Personnel reflects the approved effort. Effort for staff reflects actual effort worked during this reporting period.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: See Attached

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

Targeting Epigenetic Mechanisms in Pain due to Trauma and TBI

MR130295
W81XWH-14-1-0579

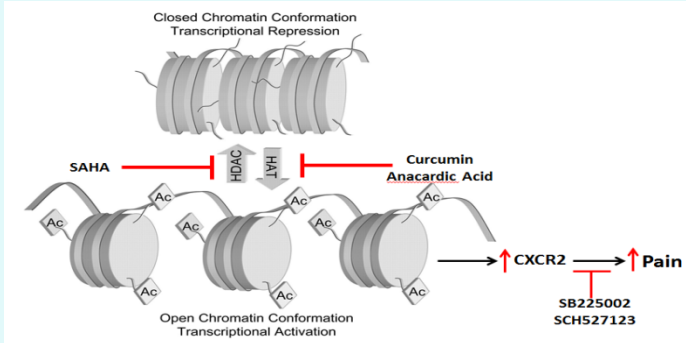
PI: David J. Clark **Org:** PAVIR **Award Amount:** $1,171,533

Study/Product Aim(s)

• To evaluate the hypothesis that histone acetyl transferase (HAT) inhibitors reduce pain and disability after surgical incision, TBI and the combination of the two injuries
•To evaluate the hypothesis that HAT inhibitors block incision-related epigenetic histone acetylation in control and TBI model animals thereby normalizing expression of key pain-related genes

Approach

The objective of this project is to define the role of agents targeting epigenetic mechanisms in reducing pain and disability after trauma. We will employ incisional and fracture extracranial injuries in conjunction with TBI. In our first aim we systematically evaluate the efficacy of HAT inhibitors in reducing pain and disability in these models. We will study the interaction of TBI with peripheral trauma. In the second aim we examine the epigenetic control of genes such as CXCR2 in controlling pain and disability. The selected test compounds will be suitable for translational human studies.



Peripheral trauma and TBI converge on spinal neuron histone acetylation to control the expression of pain-related genes including CXCR2 ultimately supporting persistent pain. Curcumin and additional pharmaceuticals can be used to regulate this process.

The required animal models, testing procedures, staff and equipment in place. Our preliminary data show that HAT inhibitors effectively reduce pain and functional disability after surgery, and that the CXCR2 chemokine receptor is involved.

Timeline and Cost

Activities / CY	14	15	16	17
Pilot studies and pre-application				
HAT inhibitors and behavioral models				
Epigenetic studies – ChIP analysis				
CXCR2 antagonists, pain and disability				
Estimated Budget ($K)	**$100**	**$395**	**$387**	**$289**

Updated: 10/27/17

Goals/Milestones

CY14 Goal – Design study, initiate experiments
- Complete pre-application process, secure animal use approvals
- Initiate pain-related testing for selected HAT inhibitors

CY15 Goals – Complete studies focused on HAT inhibitors
- Complete pain-related testing for selected HAT/HDAC inhibitors
- Initiate complex behavior-related paradigms
- Initiate epigenetic studies

CY16 Goal – Complete major epigenetic studies
- Establish that incision and TBI regulate histone acetylation
- Identify cell types in spinal cord and dorsal root ganglion tissue in which regulation is occurring

CY17 Goal – Establish pain and disability-related roles of chemokines
- Conduct pharmacological testing of selective CXCR2 antagonists in incisional, TBI and combined models.

Budget Expenditure to Date

Projected Expenditure: $1,171,533
Actual Expenditure: $886,870